Swine zoonosis risk assessment with new herd seroprofiling assays from QIAGEN

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Abstract
QIAGEN Leipzig developed the *pigtype*® product line of ELISA tests for screening for swine zoonoses. This product line now includes ELISA for detection of salmonella-, *Yersinia*-, *Trichinella*-, and *Toxoplasma*-antibodies in swine. These *pigtype* assays are validated for serum and meat juice samples and are officially approved by the German Friedrich-Loeffler-Institut. In order to follow the seroprofiling concept, the *pigtype* ELISA reagents and assay protocols are standardized. This product concept allows combining serological salmonella monitoring with serological testing for other zoonosis.

Introduction

In 2003, the European Parliament issued Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents [1]. The purpose of this directive is to ensure that zoonoses are properly monitored, and that food-borne outbreaks receive proper epidemiological investigation. The collection of this information enables the evaluation of relevant trends and sources, which therefore permits the development of effective strategies to improve the herd health level and to contribute to consumer protection.

Since 2007 Germany has implemented an official state controlled program for salmonella reduction in pork. This program is managed by the QS (Quality Scheme for Food) organization based on their salmonella Monitoring and Reduction Program for pork production[2]. This program is based on serological monitoring of pigs for antibodies to salmonella and classification according to its risk of salmonella into one of three categories[3]. One of three tests kits officially listed for this program is the SALMOTYPE® Pig Screen ELISA, which is now available as *pigtype* Salmonella Ab ELISA from QIAGEN Leipzig.

In order to allow serological monitoring for other infections, transmissible from pork to man, QIAGEN Leipzig developed ELISA test kits for detection of antibodies to *Trichinella*, *Toxoplasma*, and *Yersinia* in serum and meat juice samples. Those assays are fully validated and approved by Friedrich-Loeffler-Institute in Germany and harmonized, regarding reagents and protocols.

The purpose of this study was to evaluate the performance of new swine zoonosis assays in service and research laboratories based on well characterized samples. Furthermore, prevalence data for different zoonoses were obtained.

Material and Methods

Sets of 8 serum samples per zoonosis parameter were sent to 6 laboratories in Germany and Austria. The samples (each vial 50 µl) were shipped frozen to the participants and storage instructions requested the samples to be stored at –20°C until analysis.

The analysis of the samples was performed blinded. The samples were identifiable by sample ID numbers (#1 – #8) with the prefix SM (= salmonella), TOX (= *Toxoplasma*), TR (= *Trichinella*), and YS (= *Yersinia*), respectively.

In Table 1, the samples of each test panel are listed, supplemented with information to sample origin and status. The status of field sera refers to results determined during validation of the respective *pigtype* ELISA kit. Samples were sourced from field studies, the School of Veterinary Medicine of the Leipzig University, and the Federal Institute of Risk Assessment in Germany.

The participant laboratories were requested to test the samples in two runs (e.g., on separate days) according to manufacturer's instructions.

Two data points per sample and laboratory were generated, 12 data points per sample, 96 data points per assay, and 384 data points for the inter-lab trial samples sent.

Table 1. Description of the test panel samples for salmonella (= SM), *Toxoplasma* (= TOX), *Trichinella* (= TR), and *Yersinia* (= YS).

Sample ID	Origin		
SM-1	Salmonella field serum swine: weak positive	TOX-1	*Toxoplasma gondii* field serum wild boar: positive
SM-2	Salmonella field serum swine: strong positive	TOX-2	*Toxoplasma gondi*i field serum wild boar: weak positive
SM-3	Field serum swine: negative	TOX-3	*Toxoplasma gondii* field serum sheep: positive
SM-4	Salmonella field serum swine: positive	TOX-4	Field serum swine: negative
SM-5	Salmonella field serum swine: strong positive	TOX-5	Field serum swine: negative
SM-6	Salmonella field serum swine: weak positive	TOX-6	Field serum swine: negative
SM-7	Field serum swine: negative	TOX-7	Field serum swine: negative
SM-8	Serum of a *Salmonella Typhimurium* vaccinated 84-day-old swine (21 d.p.i.; 3rd vaccination): positive	TOX-8	*Toxoplasma gondii* field serum wild boar: weak positive
TR-1	*Trichinella* field serum wild boar: positive	YS-1	*Yersinia* field serum swine: positive
TR-2	Field serum swine: negative	YS-2	Field serum swine: negative
TR-3	Field serum swine: negative	YS-3	Serum of a *Yersinia enterocolitica* experimentally infected 54-day-old swine (26 d.p.i.): strong positive
TR-4	*Trichinella* field serum wild boar: strong positive	YS-4	Serum of a *Yersinia enterocolitica* experimentally infected 41-day-old swine (13 d.p.i.): weak positive
TR-5	Serum of a *Trichinella spiralis* experimentally infected swine (20 w.p.i.): positive	YS-5	Field serum swine: negative
TR-6	Field serum swine: negative	YS-6	Serum of a *Yersinia enterocolitica* experimentally infected 51-day-old swine (23 d.p.i.): positive
TR-7	*Trichinella* field serum wild boar: positive	YS-7	*Yersinia* field serum swine: weak positive
TR-8	Field serum swine: negative	YS-8	Serum of a *Yersinia enterocolitica* experimentally infected 58-day-old swine (30 d.p.i.): strong positive

In addition, one participating laboratory tested serum from 32 fattening pigs, 20 organic pigs, and 25 wild boar samples from their sample collection.

QIAGEN Leipzig *pigtype* ELISA test kits were sent to each of the participants of the inter-lab trial. All laboratories received ELISA test kits with the same batch number (Table 2).

Table 2. ELISA test kits and associated batch number.

Test kit name	Batch No.
pigtype Salmonella Ab	11-11.2SM
pigtype Toxoplasma Ab	12.02.1TOX
pigtype Trichinella Ab	12-03.1TR
pigtype Yersinia Ab	12-01.1YS

The *pigtype* Salmonella Ab is an updated version of the well-known SALMOTYPE PigScreen ELISA. The assay detects antibodies to salmonella serotypes of group B, C, D, and E (Kauffmann-White-scheme) in porcine serum, plasma, and meat juice samples.

pigtype Yersinia Ab is an ELISA for detection of antibodies to pathogen *Yersinia* strains in porcine serum, plasma, and meat juice samples.

pigtype Toxoplasma Ab and *pigtype* Trichinella Ab are multi-species ELISA test kits for detection of antibodies to *Toxoplasma gondii* and *Trichinella* spp., respectively. The kits are capable of detecting their respective pathogens in porcine serum, plasma, and meat juice samples. Furthermore, the kits are capable of detecting their respective pathogens in serum

and plasma samples of different mammalian species such as fox, goat, sheep, cattle, horse, dog, and/or cat.

All the ELISA products have interchangeable sample dilution buffer, wash buffer, substrate-, and stop-solutions. They use the same test procedure steps, with a 60 minute sample incubation, 30 minute conjugate incubation, and 10 minute substrate incubation. All steps are performed at room temperature. The test procedures are flexible with the option of a 60 minute incubation period at room temperature or an overnight incubation at 2–8°C (ON protocol).

Results

All laboratories scored the correct results for negative and positive samples using the *pigtype* Salmonella Ab (Figure 1). The total CV for positive samples of the test panel, using *pigtype* Salmonella Ab, is 12.7%. Using *pigtype* Toxoplasma Ab, all laboratories scored the correct results. The total CV for the positive samples of the test panel, using *pigtype* Toxoplasma Ab is 13.5% (Figure 2).

Figure 1. *pigtype* Salmonella Ab box plot results.

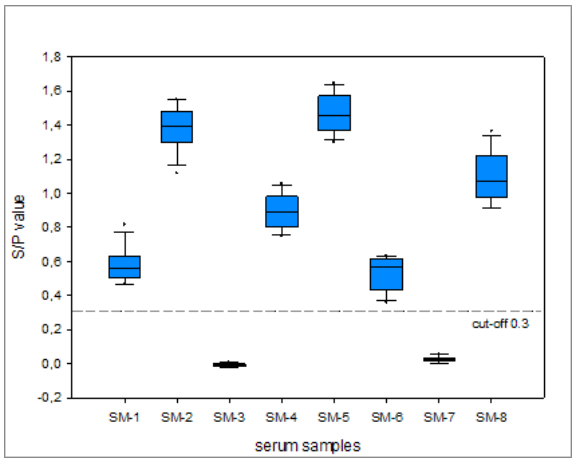
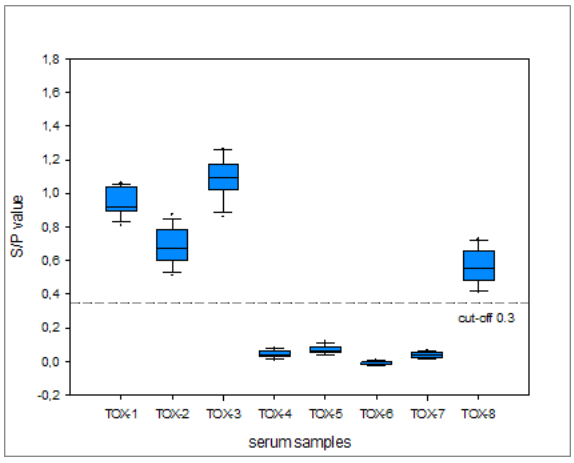


Figure 2. *pigtype* Toxoplasma Ab box plot results.



All laboratories scored the correct results using *pigtype* Trichinella Ab,. The total CV for the positive samples of the test panel, using *pigtype* Trichinella Ab is 8.8% (Figure 3). Using *pigtype* Yersinia Ab, all laboratories scored the correct results. The total coefficient of variation is 8.8% for the positive samples of the test panel in the *pigtype* Yersinia Ab (Figure 4).

Figure 3. *pigtype* Trichinella Ab box plot results

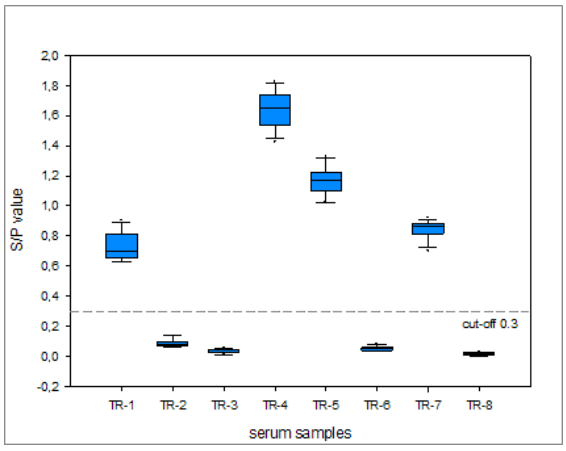
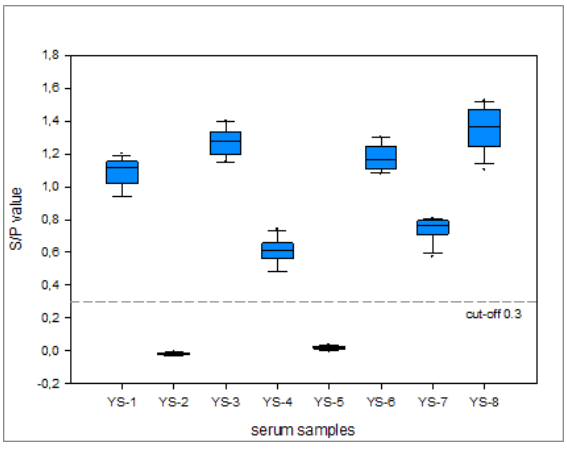
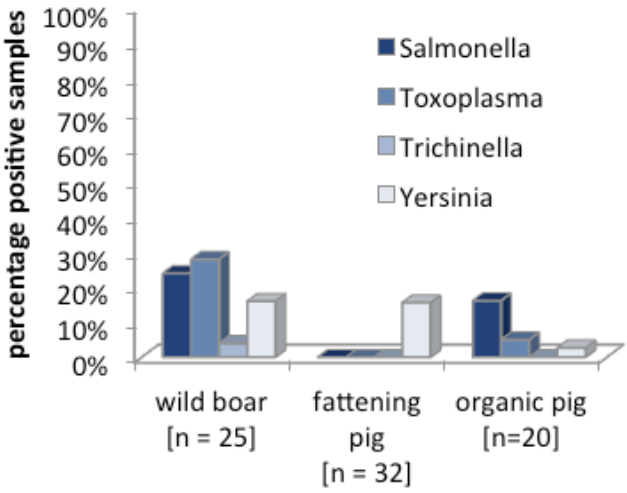


Figure 4. *pigtype* Yersinia Ab box plot results



As part of this study a limited number of field samples were tested. In fattening pigs a low percentage of *Yersinia* antibody positives were detected, while in organic pigs more salmonella and *Toxoplasma* antibody positives were found. The highest percentage of salmonella, *Toxoplasma*, and *Yersinia* positives was detected in wild boars. 4% of tested wild boar sera had antibodies specific to *Trichinella* (Figure 5).

Figure 5. Prevalence of antibodies to salmonella, *Toxoplasma*, *Trichinella*, and *Yersinia* in wild boar, fattening pigs, and organic pigs.



All of the participants were comfortable with sample dilution, quantity of reagents, and duration of the test. Two laboratories performed the ELISA manually and 4 laboratories semi-automated (no fully automated testing). There was little inter-lab variation with each sent ELISA. None of the laboratories reported any problems in the procedure of the tests.

Conclusions

In this study, there was a ≥99% agreement of the test panel results for the participating laboratories. Our data suggest the suitability of the *pigtype* assays for an easy-to-use and cost-efficient serological monitoring for zoonotic diseases in swine herds. This could be an effective tool to use, under the European Directive on zoonoses, and bring improvements to herd risk assessment and risk oriented meat control.

References

1.) **DIRECTIVE 2003/99/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC**Directive 2003/99/EC

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF

2.) **Guideline Salmonella Monitoring and Reduction Program for Pork Production, www.q-s.de/dc_salmonella_programme_pig_lab.html**

3.) **Blaha T. (2004), Up-to-date information from the German QS salmonella monitoring and reduction programme. Dtsch Tierarztl Wochenschr. Aug; 111(8):324-326.**